Positive reinforcement training: a tool for care and management of captive vervet monkeys (*Chlorocebus aethiops*)

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Abstract

In modern zoos, training should be an integral component of the animal care and management. The benefits of training include the opportunity for positive interactions with caretakers. This study was carried out with a group of vervet monkeys *(Chlorocebus aethiops)* housed at the Garda Zoological Park. Using focal animal sampling, we observed the behaviour performed by all group members from December 2007 to August 2008. The group took part in a training programme to be isolated in a familiar area before the subjects were included in a cognitive study. We collected behavioural data during a pre-training period to assess the social behaviour of the colony and during the training period to investigate the effects of the training programme on the behaviour of individuals. Additionally, a second phase of the study was conducted and training sessions with individual monkeys were video-recorded to determine the behaviour of animals during each training session and thus to confirm that they were suitable for participating in the procedure. Our results suggest that the training programme enriched the daily routine of these captive primates by increasing affiliative behaviours while decreasing agonistic behaviours. Furthermore, there was behavioural response variability among the individuals under training procedure. However all the individuals were trained to calmly enter in a familiar area and to be isolated from other members of the group. In conclusion, our findings highlight the importance of using the positive reinforcement training to reduce the tension directly associated with potentially stressful procedures by allowing primates to voluntarily participate in these procedures. In addition, the training was found to be an enrichment tool for vervet monkeys.

Keywords: animal welfare; captivity; enrichment tool; husbandry refinement; operational conditioning; stress

Introduction

In the early 1900s, Skinner suggested that the best way to understand animal behaviour is to look at the causes of an action and its consequences. This approach is called "operant conditioning" (Skinner 1981), and it entails the changing of behaviour by use of reinforcement, which is provided after the desired response. The Skinner theory was based on the "Law of Effect" (Thorndike 1911) with the addition of a new term, "reinforcement," emphasizing that behaviour that is reinforced tends to be repeated (i.e., strengthened).

Operant conditioning techniques can be applied successfully to improve the behavioural management of nonhuman primates in research settings (Owen & Amory 2011). It is essential that zoo curators review the literature to assess objectively whether specific positive reinforcement training methods may enhance captive management and research procedures with the animals (Schapiro et al 2003).

Operant conditioning with positive reinforcement has been shown to be the optimal tool for training captive primates to calmly enter an experimental/training area while remaining isolated from the rest of the group, thus achieving the voluntary cooperation of individuals in cognitive research (Desmond & Laule 2005; Prescott & Buchanan-Smith 2003). These types of training programmes are used because individual primates appear to be more relaxed when they are in groups rather than isolated (Prescott & Buchanan-Smith 2003). Separating animals from their groups can be stressful, both for the individuals removed from the group and for those who remain behind. However, carrying out training sessions with primates within their social context may limit their performance (Shapiro et al 2003).

Various aspects of captive environments can increase stress levels and jeopardize the well-being of captive animals. The use of positive reinforcement training techniques enables researchers and caretakers to reduce the tension associated with potentially stressful procedures and situations (Carlstead 2009). The role of training in the management of captive populations has changed significantly over time, and it has evolved into a series of techniques that allow for

medical treatment and behavioural research and improve animal welfare (Laule 1993). As a method of training captive animals, operant conditioning with positive reinforcement is a practice that is increasingly recognized by zoos as a valuable addition to standard husbandry and behavioural management methods (Crowell-Davis 2008; Fuller et al 2012; Laule 2003). Animal training is effective not least because the animals themselves contribute to an improvement of their own handling free of stress (Colahan & Breder 2003).

Positive reinforcement training improves care and reduces stress by enlisting a primate's voluntary cooperation with targeted activities, including husbandry and cognitive research activities (Laule & Whittaker 2007; Pomerantz & Terkel 2009). Although training should not be the only form of enrichment, it can be an integral part of any enrichment programme (Mellen & Mac Phee 2001). Recent studies (Mattison 2012; Owen & Amory 2011) indicated that the use of positive reinforcement training considerably reduced the potential for stress and improved welfare during the capture and containment of New World monkeys.

Husbandry training is widespread in zoos and often considered helpful as environmental enrichment technique (Melfi 2013). However, although several studies assessed the effect of environmental enrichment programmes on animal behaviours, few studies empirically evaluated the impact of training on animal welfare - especially outside the training sessions (reviewed in Melfi 2013).

The purpose of the present study was to evaluate whether a training programme for vervet monkeys (*Chlorocebus aethiops*) could be used to induce them to cooperate in behavioural management (i.e., to elicit voluntary participation in routine husbandry, animal transport, and health-care procedures). Specifically, the study aimed to detect if the monkeys could be trained to be isolated in a familiar area. In addition, we tried to assess whether this training programme is a multifunctional tool that can be used to create a variety of enrichment opportunities for captive animals.

Materials and Methods

A group of ten (four males and six females) vervet monkeys (*Chlorocebus aethiops*) housed at Garda Zoological Park (Italy) was involved in the training programme **(Table 1)**. The vervet monkeys were trained to be isolated in the training area, while the other members of the colony remained in the indoor enclosure without visual or olfactory contact with the isolated individuals. A positive reinforcement technique (Schapiro et al 2003), using guillotine doors, was employed to train the subjects to calmly enter the experimental/training area in order to achieve their voluntary cooperation in a problem-solving study.

----------Table 1----------

The training area consisted of a 10-m^2 tunnel linking the 29-m^2 indoor with the 419-m^2 outdoor enclosure, whereas the training apparatus was an open rectangular wooden box hanging in the tunnel.

Before the training session, the individuals were habituated to stay in the tunnel by providing them with food in the apparatus, then the individuals were separated by the group by closing the guillotine doors dividing the tunnel from indoor and outdoor enclosures. During the training session, if entered successfully the tunnel each subject could take the reward from the apparatus. This reward was a cube (1.5x1.5 cm) of jelly for primates consisting of a mixture of vegetables, fruits and nuts ("Delicacy Gelée" supplied by Viten®, Udine, Italy). ~~The training sessions lasted differently (but never more than three minutes each), on the basis of the emotional state of each individual.~~The length of each training session varied (but never exceeded 3 minutes) depending on the emotional state of each individual.

In the first phase of the study we used focal animal continuous sampling to assess the behaviour of the subjects within their social context (Altmann 1974). Each animal was observed during 15-minute sessions in three different periods (each period made by ten sessions per subject for a total of 100 sessions) for a total of 75 hours: the 'baseline' before the training period, the 'first

period' once training had begun, and the 'second period' during the training. In the so-called 'first period' and 'second period' we observed the animals during 15-minute focal animal sessions immediately after each training session when they were all housed together in the outdoor enclosure; this was to investigate the effects of the training programme on their group behaviour. Each study period lasted two weeks; within each period the training sessions were always conducted at the same time of day. All individuals were tested in each session and trained spontaneously in a random order.

We collected data for social and individual behaviours. A comprehensive ethogram (Adeyemo 1997; Cheney & Seyfarth 1990; Fedigan 1972; Fedigan & Fedigan 1988) was adapted to cover a range of social and individual behaviours **(Table 2)**. Social behaviours were further grouped according to agonistic and affiliative behaviours. Agonistic behaviours included dominant and submissive behaviours. Affiliative behaviours include measures of grooming, body contact, social play, sexual behaviour, social resting and all "other affiliative" behaviours. A category designed to quantify time spent engaged in individual behaviours included self-grooming, exploration, alert, locomotion, individual play, foraging, maintenance and resting.

----------Table 2----------

The second phase of the study, consisting of additional 14 training sessions per individual, started a week after the first phase and was conducted exactly as the first one (i.e., the ten individuals were separated from their group and had to take a reward). This phase aimed at completing the isolation training process and fully prepare the animals for cognitive studies. These last sessions were video-recorded to monitor the behaviour of each individual during the isolation training session. We ~~collect~~collected data about the displacement (set of anxiety-related behaviours including actions directed toward themselves; Maestripieri 1991) of the subjects and whether or not they took the reward.

The first phase of the study, the previous three study periods, focussed on training effects on the whole colony (i.e., positive or negative effects as a result of the isolation training), whilst in the second phase the psychological well-being of the subjects during isolation and the feasibility of ~~starting the~~ problem solving trials (i.e., cognitive studies on individual and social learning which we plan to conduct) were assessed.

Data analysis of the whole study was based on duration of behaviours. We used nonparametric statistical tests (Siegel & Castellan 1992). In particular, we used the Friedman test with a series of post-hoc Wilcoxon tests with Bonferroni correction on each combination of periods (to compare the three different periods - the 'baseline' before the training period, the 'first period' once training had begun, and the 'second period' during the training). In addition, in the second phase of the study we used the Wilcoxon test to compare behaviours between the first and the last sessions. All tests were two-sided, and the significance level was set to $p < 0.05$. Analyses were performed with StatView for Windows and Macintosh (version 5.0).

Results

Observing the group behaviour, during the first phase of the study, locomotion was displayed significantly more during the "'first period'" than the "'baseline'" and the "'second period'" (Friedman test: $\chi^2 = 9.80$; $p = 0.0074$); in particular, locomotion was significantly less displayed in the 'second period' compared to the 'first period' (Wilcoxon test with Bonferroni correction: $z = -2.80$; $p = 0.0051$) **(Figure 1)** along with the progress of the training programme.

----------Figure 1----------

In order to investigate whether the training programme could be considered an enrichment tool for captive animals, we focused on social behaviour: agonistic and affiliative behaviours. In

particular, dominant behaviours were carried out most frequently during the baseline whilst gone significantly down during the "'first period'" and "'second period'" (Friedman test: χ^2 = 7.09; p = 0.0289) **(Figure 2)**. On the contrary, social resting was shown more during the "first" and "second" period than during the baseline (Friedman test: χ^2 = 6.73; p = 0.0346) **(Figure 3)**. In addition, we found no significant ~~variation~~different in other affiliative behaviours (such as grooming, body contact, social play and sexual behaviours) (Friedman tests: N.S.) when comparing the different study periods.

----------Figure 2----------

----------Figure 3----------

Furthermore, in order to assess whether a training programme for vervet monkeys could be used to induce them to cooperate in behavioural management and cognitive research, the fourteen video-recorded sessions of the second phase of the study were analysed. Comparing the first two sessions with the last two sessions of the 14 sessions, vervet monkeys showed significantly less displacement (running back and forth) during the final training sessions compared to the first sessions (Wilcoxon test: z = - 2.03; p = 0.0425) **(Figures 4.a and 4.b)**.

----------Figures 4.a & 4.b----------

Moreover, over the last two sessions of the training programme, all the monkeys took their own reward whereas in the first two sessions of the training programme only 40% of subjects ~~were calm enough to take~~took their own reward (Wilcoxon test: W = 0; p < 0.05) **(Figure 5)**. However, the number of training sessions needed to achieve the final stage (i.e., being calm and taking the reward) varied depending on each individual.

----------Figure 5----------

Discussion and Conclusion

Results of the first phase of the study, focussing on training effects on the whole colony, revealed a significant decrease in locomotion across the training sessions suggesting an improvement in the well-being of the vervet monkey, as increased locomotion have been reported as non-invasive indicator of stress in other captive primate species (Box & Rohrhuber 1993; Chamove et al 1988; Hosey & Druck 1987; Mitchell et al 1992; Schmidt 2010; Schoenfeld 1989). However, we were unable to address significant changes between the "'second period'" and the "'baseline'". As a consequence, our results for locomotion should be regarded as preliminary and more trials would be necessary to state that there is definitely an improvement of well-being. Since other behaviours (such as social behaviours, and particularly dominant behaviours and proximity between individuals) are considered to be indicators of animal welfare (Melfi & Thomas 2012), the decrease of dominant behaviours together with the increase of social resting highlighted that the training programme reduced aggressiveness and improved socialization - appearing to be an important part of environmental enrichment programmes that improve the daily routine of captive animals, as described previously by other authors (Laule et al 2003; Laule & Desdmond 1998; Laule et al 2003). Since no significant differences in other affiliative behaviours were found when comparing the baseline and the "'second period'", no negative impact on welfare as a result of the training procedure was reported (Whitehouse et al 2013). Results of this study suggest that the training programme seems to help vervet monkeys to be isolated in a familiar area and voluntarily participate in the procedure, as described previously for other primate species (Fuller et al 2012; Owen & Amory 2011; Prescott & Buchanan-Smith 2003). This can be useful for health purposes (i.e., to allow and facilitate the monitoring of vervet monkeys, by capturing and keeping without providing

stress to these animals). Thus, our results provide support for previously published findings (Carlstead 2009; Laule 2003; Owen & Amory 2011; Pomerantz & Terkel 2009) that positive reinforcement training contributes to the behavioural management and well-being of captive nonhuman primates.

Observations from the first video-recorded training sessions underlined that the individuals in the study group showed undesirable behaviours, such as displacement (Barros et al 2004; Bassett et al 2003; Kessel and Brent 2001), and did not take the reward most likely because they were not calm enough.

These findings confirmed that the isolation of a vervet monkey from his group could be stressful, especially for the individual removed from the group (Shapiro et al 2003) - this was probably due to social features of vervet monkeys (Cheney & Seyfarth 1990). However, at the end of the training procedure ~~the vervet monkeys were~~each individual was calm enough to remain isolated in a familiar area while eating a reward.

In conclusion, this study empirically evaluates the impact of training zoo animals within and outside of the training session, to fill a gap in the literature (Melfi 2013). Our results highlight that establishing a training programme might be a valuable tool that can be used to accustom captive vervets to isolation in a familiar area through positive reinforcement. In addition, the positive reinforcement training could also be used as valuable tool for an enrichment programme addressing elements of well-being for captive primates. Future research work should examine the effect of husbandry training techniques on abnormal behaviours, activity budget and proximity between individuals. In addition, we focused on the behavioural approach but we neglected the endocrine component. However, the ability to collect and analyse both physiological and behavioural data is crucial for evaluating the stress responses and welfare of animals in captivity (e.g., Peel et al 2005); in particular, one useful indicator of stress is the measurement of cortisol levels, whereas little is known about how testosterone and progesterone vary in stressful situations - such as cases of isolation (Fontani et al 2014). Actually, to assess the impact of stress comprehensively, multiple components of the stress response (i.e.,

behavioural, hormonal, and immunological factors) should be monitored, and the links among these components should be considered as well (Peel et al 2005).

Animal welfare implications

Results show reduction in aggressive behaviours and increase of positive social interactions, suggesting that the training can also be used as husbandry refinement. Indeed, the positive reinforcement training provides animals with wider choice and control over their lives. Furthermore, to train animals in order to voluntarily enter into a familiar area and be isolated from other group members might support these animals to voluntarily cooperate in husbandry and veterinary procedures. Therefore, it might contribute to decrease both the use of anesthesia and the stress for trained animals and the entire group as well (Laule et al 1992; Luttrell et al 1994, Veeder et al 2009).

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Figure 1 - Locomotion (N=10). Comparison of the locomotion across the three study periods (baseline, first period, second period); error bars stand for the standard deviation; locomotion, first period vs. second period (Wilcoxon test with Bonferroni correction: $z = -2.803$; $p = 0.0051$).

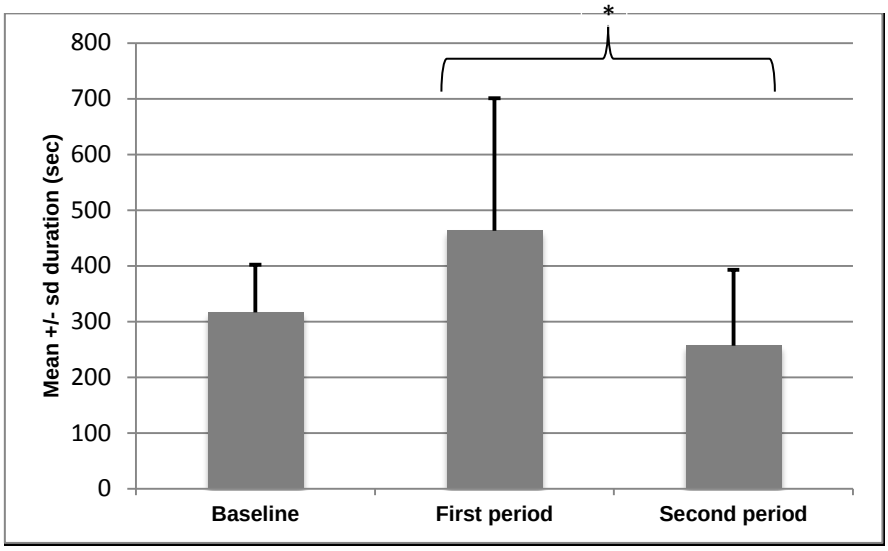

Figure 2 - Dominance and submission (N=10). Comparison of dominant and submissive behaviors across the three study periods (baseline, first period, second period); error bars stand for the standard deviation; dominant behaviors, baseline vs. second period (Friedman test: $\chi^2 = 7.091$; $p = 0.0289$).

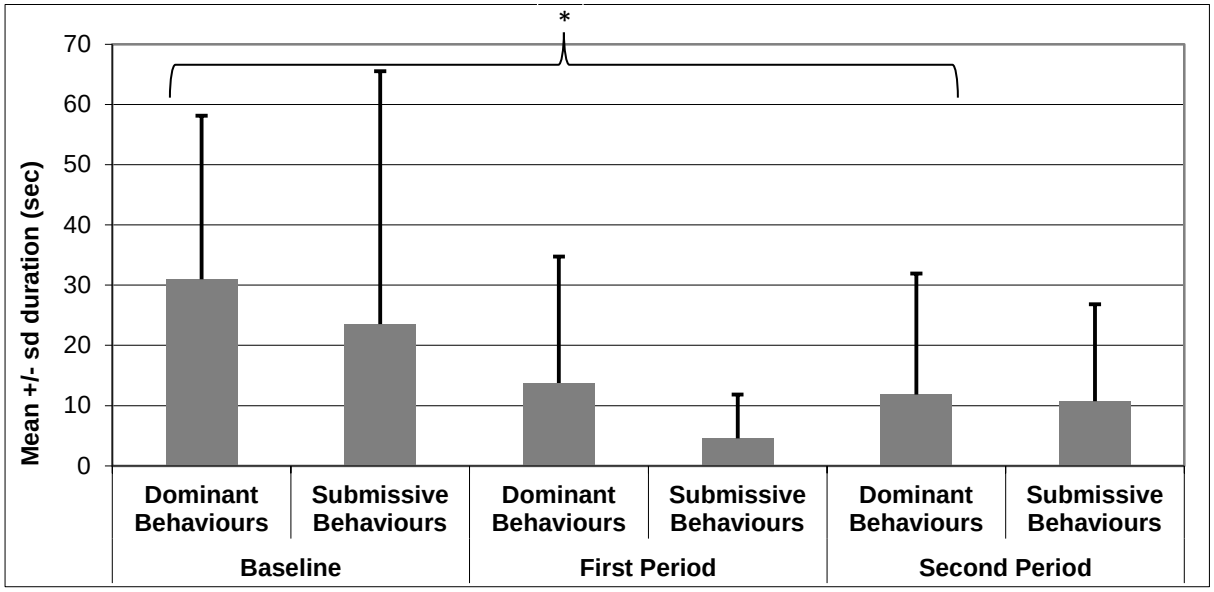

Figure 3 - Social resting (N=10). Comparison of social resting across the three study periods (baseline, first period, second period); error bars stand for the standard deviation; social resting, baseline vs. first and second period (Friedman test: $\chi^2 = 6.727$; $p = 0.0346$).

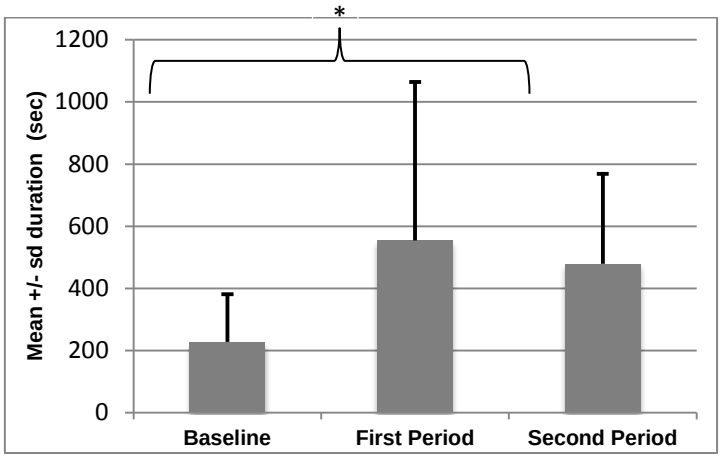

Figure 4.a - Displacement (N=10). Comparison of the displacement (running back and forth), between final training sessions and first sessions, error bars stand for the standard deviation; running back and forth, first sessions vs. last sessions (Wilcoxon test: z = - 2.028; p = 0.0425).

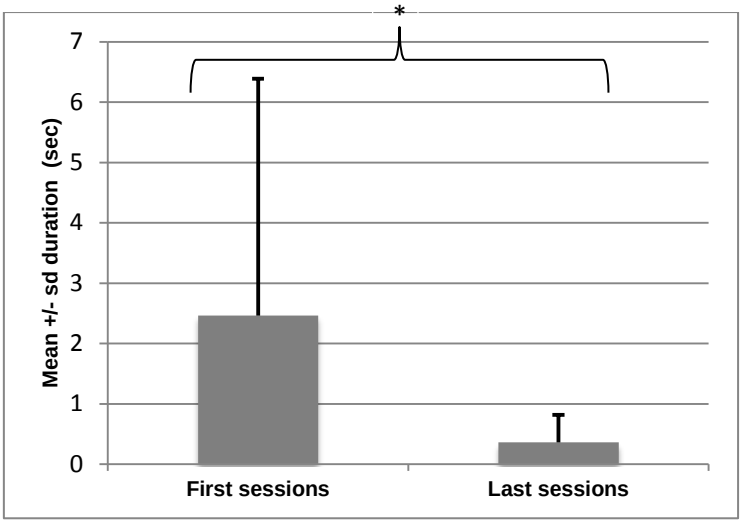

Figure 4.b - Displacement by individuals. Comparison of the displacement (running back and forth), between final training sessions and first sessions, by single individuals.

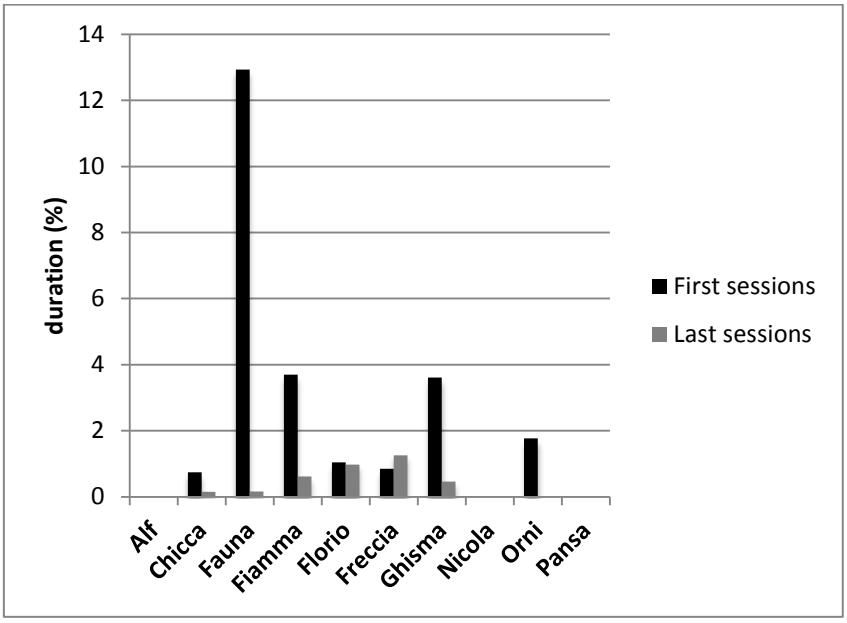

Figure 5 - Collection of rewards. Comparison between the first and last two sessions of the training programme, in terms of individuals calmly taking their own reward (Wilcoxon test: $W = 0$; $p < 0.05$).

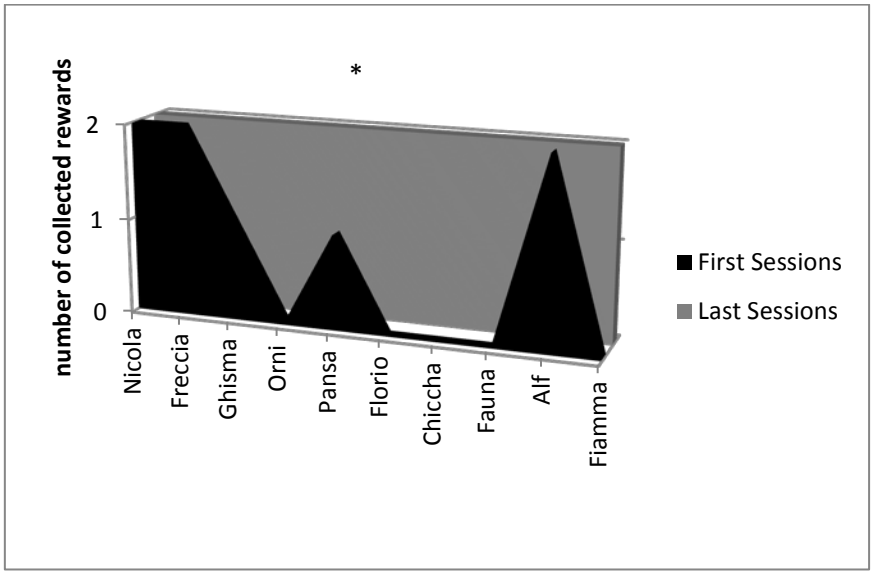

Table 1 - Group size (N=10) and composition (sex and dominance rank) housed at Parco Natura Viva-Garda Zoological Park at the beginning of the study (June 2008).

Name	Sex	Age (years)
Alf *	Male	15
Fauna	Female	12
Orni	Female	12
Fiamma	Female	10
Ghisma	Female	9
Freccia	Female	7
Nicola **	Male	3
Florio	Male	2
Chicca	Female	2
Pansa	Male	1

* alpha

** beta

Table 2 - Ethogram, based on previous comprehensive ethogram (see Adeyemo 1997), modified to cover a range of social and individual behaviours.

Behavioural class	Behavioural subclass	Behavioural category		Description
Social behaviours	Agonistic behaviours	Dominant behaviours	Dominance with conflict	Aggression toward an individual (hit, fight, bite, etc.)
			Dominance without conflict	Hugging an individual's back without copula, others actions that express dominance toward an individual, different from the aggression
			Dominance without submission by the receiver	The individual receiving threats or aggression does not display submissive behaviour
			Redirected aggression	An individual who received aggression from a second individual is aggressive toward a third individual not involved in the conflict
		Submissive behaviours	Submission with conflict	An individual shows submissive behaviour (crouching, sexual presentations, showing the back, fear expressions, escaping from an individual) after a physical aggression
			Submission without conflict	An individual shows submissive behaviour (crouching, sexual presentations, showing the back, fear expressions, escaping from an individual) but no physical aggression was present
	Affiliative behaviours	All other affiliative behaviours		All other affiliative behaviours not included in the ethogram
		Body contact		Being in contact with other individuals with attention to the surrounding environment
		Grooming		Cleaning another individual's fur with hands or mouth
		Sexual behaviours		Receiving or doing ano-genital inspection
		Social play		Non-agonistic interaction: fight-play, somersaults, chase
		Social Resting		Resting in contact with other individuals

Individual behaviours	Alert	Looking around carefully to detect potentially dangerous situations
	Exploration	Investigating and examining different areas of the enclosure and environmental enrichments
	Foraging	Search and ingestion of food available in the enclosure
	Individual play	Playing with objects or interacting with the environment
	Locomotion	Walking, running, climbing
	Maintenance	Eating, drinking, urinating, defecating
	Resting	Resting alone
	Self-grooming	Cleaning one's self fur with hands and mouth, sexual self-inspection, scratching